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14. ABSTRACT

This proposal is driven by hypothesis that certain biological factors associated with metabolic syndrome may play a role in racial differences in prostate cancer (PCa) aggressiveness and prognosis. This is the first study to include large numbers of African American men in an evaluation of metabolic syndrome and the first to study the association between metabolic syndrome and PCa recurrence by race. Our hypothesis is being tested in 2 Aims: 1) To quantitatively assess levels of multimeric adiponectin complexes and selected biochemical markers related to inflammation, insulin resistance and oxidative stress in serum of newly diagnosed PCa patients with non-aggressive and aggressive disease, and examine racial differences in statistical correlations between these biomarkers and adiponectin in PCa patients with aggressive disease; and 2) determine if there are differences in single nucleotide polymorphisms (SNPs) in selected candidate genes implicated in metabolic syndrome, obesity, chronic inflammation inflammation, and oxidative stress in PCa patients with aggressive and non-aggressive disease, and determine how these differences predict the risk of aggressive PCa and disease recurrence by race. We predict that this work should identify novel biomarkers for detection and prognosis of aggressive PCa and provide basis for future studies of mechanisms that drive racial disparities in PCa aggressiveness and outcome.

15. SUBJECT TERMS
prostate cancer, metabolic syndrome, African American, racial disparity, biochemical markers, single nucleotide polymorphisms

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INTRODUCTION:

Emerging data suggest that obesity and metabolic syndrome contribute to an increased risk of prostate cancer [1-8]. African American men are predisposed to specific features of metabolic syndrome, such as hypertension and abdominal obesity, which are also risk factors for PCa in this racial group [9, 10]. The proposed studies will test the hypothesis that specific biochemical and genetic factors related to inflammation, insulin resistance and oxidative stress, contribute to racial/ethnic disparity in aggressiveness and recurrence of PCa. Predisposition of African Americans to hypertension and vascular dysfunction has been linked to suppressed levels of the hormone adiponectin and increased levels of markers of systemic inflammation [11, 12]. Recent data also suggest that distribution of different multimeric forms of adiponectin (as opposed to total protein levels) may be reflective of insulin resistance and oxidative stress [13, 14]. This suggests that certain biological factors associated with metabolic syndrome may play a role in racial differences in PCa aggressiveness and prognosis.

The present study is based on the **hypothesis** that specific biochemical and genetic factors contribute to racial/ethnic disparity in aggressiveness and recurrence of PCa. **Our specific aims are to:** 1) quantitatively assess levels and distribution of multimeric adiponectin complexes and selected biochemical markers related to inflammation, insulin resistance and oxidative stress in serum of newly diagnosed PCa patients with non-aggressive and aggressive disease, and examine racial differences in statistical correlations between these biomarkers and adiponectin in PCa patients with aggressive disease; and 2) determine if there are differences in single nucleotide polymorphisms (SNPs) in selected candidate genes implicated in metabolic syndrome, obesity, chronic inflammation, and oxidative stress in prostate cancer patients with aggressive and non-aggressive disease, and determine how these differences predict the risk of aggressive prostate cancer and disease recurrence by race. To achieve these aims we are: 1) utilizing Adiponectin (Multimeric) Enzyme Immunoassays for quantitative assessment of levels and distribution of multimeric forms of adiponectin in serum; 2) assessing levels of 15 biomarkers implicated in inflammation, insulin resistance and oxidative stress using Custom Quantitative Antibody Arrays; 3) examining the statistical relationships between adiponectin and these biomarkers in predicting racial differences in prostate cancer aggressiveness and prognosis using scatterplots as a statistical graphic, and Pearson product moment correlation coefficients as measures of the strength of linear association; 4) performing SNP analyses and assessments of allele frequency differences in 34 genes involved in various pathways including obesity, inflammation, insulin resistance and oxidative stress using Illumina GoldenGate assays; and 5) employing a logistic regression model to estimate the risk of aggressive prostate cancer associated with each genotype by race, and a Cox Proportional hazards modeling approach to estimate the risk of recurrence associated with each genotype by race.

We predict that these studies will confirm our hypothesis that metabolic syndrome-induced inflammation, insulin resistance and oxidative stress differentially influence disease progression in African American and European American men. This work should identify novel biomarkers for detection and prognosis of aggressive prostate cancer. Results of this study will provide the basis for future validation of these biomarkers as biological targets for improved therapy and/or prevention of aggressive disease, and studies of novel signaling pathways and the molecular mechanisms that contribute to aggressiveness of prostate cancer.

BODY:

In year three of this study, we continued to recruit patients and collect samples for the serum and SNP analyses. We have reached a target of 500 patients at the end of year 2; however, some of the patients turned out to be ineligible, or their specimens were not collected (survey only patients) – thus

recruitment continued thru the year 3 of this study. For Task 1, only cases diagnosed on or after September 1st, 2009 (newly diagnosed patients) are being included in the analysis. For the genomic DNA analyses described in Task 2, all patients recruited into PC081618 (i.e., prostate cancer cases diagnosed on or after January 1st, 2004) are eligible for inclusion. Current status of our recruitment after eliminating ineligible specimens is shown in Table 1. Although we have now exceeded the goal of 500 patients, the newly diagnosed cases are below a target of 45 for European American (EA) patients

Table 1. Prostate cancer patients enrolled in the metabolic syndrome investigation to date

	Newly Diagnosed		Existing		Total
	AA	**EA**	AA	EA	
Enrolled-complete	47	18	226	126	417
Enrolled-survey only	8	4	38	25	75
Enrolled-blood only	4	1	6	3	14
Consented only	2	0	8	2	12
Total	61	23	278	156	518
Withdrew	1	0	3	1	5

AA: African American
EA: European American

We have requested and have been granted a 3-month no-cost extension to finalize sample analyses and to perform biostatistical assessments and comparisons. The final results will be submitted in the final report, which is due to the Agency by October 31, 2013. In the present report below we will just briefly discuss the current status of our analyses at the end of year 3 of the grant.

TASK 1: We will quantitatively assess levels and distribution of multimeric adiponectin complexes and selected biochemical markers related to inflammation, insulin resistance and oxidative stress in serum of newly diagnosed PCa patients with non-aggressive and aggressive disease, and examine statistical correlations between these biomarkers in predicting racial differences in PCa aggressiveness and prognosis.

Sub-Aim 1A: To explore biochemical markers that predict PCa aggressiveness, we will quantitatively assess serum levels of 15 cytokines related to inflammation, insulin resistance and oxidative stress and correlate them with levels and distribution of multimeric complexes of adiponectin in non-aggressive (n=45) and aggressive (n=45) patients).

Sub-Aim 1B: To explore racial differences in aggressive PCa we will determine cytokine levels and their correlations with adiponectin complexes in AA (n=45) vs. EA (n =45) men with aggressive disease. We will further stratify the results based on prevalence of metabolic syndrome to assess the differential influence of metabolic syndrome-induced inflammation, insulin resistance and oxidative stress on disease progression between AA and EA

We have reported preliminary findings of cytokine arrays and Adiponectin ELISA analyses in the last year's report. We have processed the rest of qualified specimens and biostatistical analyses are in progress by our qualified biostatistician on this project. Some of the cytokine array results are also currently being validated by ELISA analyses.

TASK 2: We will determine if there are differences in the single nucleotide polymorphisms (SNPs) in selected candidate genes implicated in metabolic syndrome, obesity, chronic inflammation, and oxidative stress in PCa patients with aggressive and non-aggressive disease, and determine how these differences predict the risk of aggressive PCa and disease recurrence by race.

Sub-Aim1: To explore genetic markers that may predict PCa aggressiveness, we will examine differences in allele frequency in 34 genes involved in various pathways I ncluding obesity, inflammation, insulin resistance and oxidative stress between PCa cases diagnosed with aggressive disease and cases considered non-aggressive.

Sub-Aim 2: To explore genetic markers that may predict PCa recurrence, we will examine differences in allele frequency in 34 genes involved in various pathways including obesity, inflammation, insulin resistance and oxidative stress between prostate cancer cases with evidence of disease recurrence and non-recurring cases.

We proposed to perform this analysis on all 500 DNA samples collected for this study. Therefore it was necessary to wait until DNA from all eligible patients was collected, purified and prepared for analyses. A list of 1536 TagSNPs within the genes from the pathways of interest was prepared using Tagger <http://www.broadinstitute.org/mpg/tagger/>. Genotype of tagSNPs was determined using a custom panel run on the Illumina GoldenGate® genotyping platform. These analyses were recently completed, and the results are currently being analyzed by our experienced biostatistician on this project. We predict that these pathways influence prostate cancer progression and therefore would predict aggressive disease and prognosis.

KEY RESEARCH ACCOMPLISHMENTS:

- We have now completed the recruitment of PCa patients to this study
- The percentage of participation for African American patients is high (62%), which gives us a unique opportunity to study the mechanisms behind PCa aggressiveness and potential for recurrence in this population
- Studies of serum markers of newly diagnosed patients are completed and biostatistical analyses are in progress
- The tagSNP analyses using DNA samples from all patients recruited to this study have been completed using custom Illumina GoldenGate® panel of 1535 TagSNPs. Biostatistical analyses are in progress.
- Final results of this study will be included in the Final report, which is due to DOD by October 31, 2013.

REPORTABLE OUTCOMES:

1. Invited Lectures/Presentations at National/International Meetings

A. "Protease-adipokine signaling in bone metastasis." **Gordon Research Conference on Proteolytic Enzymes and Their Inhibitors.** Lucca, Italy, May 2-7, 2010.

B. "Delayed Progression of Prostate Bone Tumors in Cathepsin K-deficient Mice: Novel Proteolytic Pathways and Adipocyte Involvement in the Bone Microenvironment**." Department of Defense Prostate Cancer IMPaCT Conference**, Orlando Florida, March 9-12, 2011

2. Invited/Refereed Presentations at Local/Regional Meetings

A. "Exploring the Links between Obesity, Inflammation, Metabolic Syndrome and Prostate Cancer". **Presentation for Carla G. Hawley-Bowland, Medical Corps General**, during her visit related to Army Physician in Training Program, October 5, 2010.

B. "Proteolytic and Inflammatory Pathways in Bone Metastasis: Lessons From Cathepsin K Knockout Mice." **Joint Retreat of Proteases and Cancer and Breast Cancer Programs at Karmanos Cancer Institute**; Detroit, MI, December 14, 2010.

C. "Biochemical markers of inflammation and racial disparities in prostate cancer" **Karmanos Cancer Institute Ground Rounds**, November 3, 2011.

D. "Adipocytes, bone marrow inflammation, and progression of prostate tumors in bone: Does cathepsin K play a role? **University of Toledo, Department of Pharmacology seminar series**, November 9, 2011

E. "Exploring inflammatory pathways linking metabolic syndrome with racial disparities in prostate cancer", **Karmanos Cancer Institute Research Retreat**, May 9, 2012

F. "Adipocytes, bone marrow inflammation, and progression of prostate tumors in bone"; University of Michigan, July 9, 2012

G. *"*Combination of Imaging and Genomic Approaches to Identify Bone and Tumor Responsesto RTK-Targeted Therapy for Metastatic Prostate Cancer". **National Oncogenomic and Molecular Imaging Center (NOMIC) Retreat,** Detroit, MI, February 15, 2013

H. "Metabolic adaptation in prostate cancer growth and survival in bone: does bone marrow fat play a role?" **Wayne State University, Department of Pathology,** March 13, 2013

I. "The Evolving Role of Tumor Associated Macrophages in Prostate Cancer Therapy". **Prostate Cancer Research Team** seminar, June 21, 2013

J. "Functional roles of bone marrow adipocytes and macrophages in metastatic prostate cancer growth and survival in bone". **Prostate Cancer Research Team** retreat; June 26, 2013.

3. Abstracts:

A. Sreeker Reddy, Mackenzie Herroon, and **Izabela Podgorski**. Analyzing the Effects of Adipocyte Conditioned Media on Bone Metastatic Cell Lines. **Summer Undergraduate Student Poster Day**, Wayne State University, August 10, 2010.

B. **Izabela Podgorski,** Mackenzie Heroon, Deborah Rudy, Anju Mukundan, and Craig Giroux. Delayed Progression of Prostate Bone Tumors in Cathepsin K-deficient Mice: Distinct Effects on Tumor- and Host-Initiated proteolytic Pathways. **Metastasis Research Society and AACR Joint Conference on Metastasis and Tumor Microenvironment.** Philadelphia, PA, September 12-15, 2010.

C. Aimalie Hardaway, Katelyn Powell, Mackenzie Herroon, and **Izabela Podgorski**. "Role of adipocyte-derived factors in prostate cancer bone metastasis." **Wayne State Graduate Exhibition**, March 2nd, 2011, **2nd place winner**.

D. **Izabela Podgorski**, Mackenzie Herroon, Craig Giroux, James Grannneman, Deborah Rudy, Anju Mukundan. Delayed Progression of Prostate Bone Tumors in Cathepsin K-deficientMice: Novel Proteolytic Pathways and Adipocyte Involvement in the Bone Microenvironment**. Department of Defense Prostate Cancer IMPaCT Conference,** Orlando Florida, March 9-12, 2011; **Abstract selected for podium presentation.**

E. Jennifer Beebe-Dimmer, Cathryn Bock, **Izabela Podgorski**, Susan Bolton, Sarah Lewis, and Isaac Powell. The Influence of Metabolic Syndrome on Prostate Cancer Progression and Risk of recurrence in African American and European American Men" **Department of Defense Prostate Cancer . IMPaCT Conference**, Orlando Florida, March 9-12, 2011.

F. Aimalie L. Hardaway***,** Katelyn A. Powell*, Mackenzie K. Herroon, Erandi N. Rajagurubandara, and **Izabela Podgorski** . "Inflammation and Oxidative Stress in Prostate Cancer Bone Metastasis: Contribution of Bone Marrow Adipocytes"; **KCI Proteases and Cancer Program Annual Retreat,** December, 2011

G. Aimalie L. Hardaway*, Mackenzie K. Herroon, Erandi N. Rajagurubandara, Gorica Ristic*, and **Izabela Podgorski.** "Inflammation and Obesity in Prostate Cancer Bone Metastasis: Role of Bone Marrow Macrophages". **3rd Annual Graduate Exhibition,** March 6, 2012

H. Aimalie Hardaway*, Mackenzie, K Herroon, Erandi Rajagurubandara, Audrey Turchick*, and **Izabela Podgorski.** The Role of Bone Marrow Adipocyte-Induced HO-1 Expression Prostate Tumor Survival in Bone. **AACR Annual Meeting,** Chicago, IL, March 31- April 4, 2012.

I. Hardaway, A.*, Herroon, M., K., Rajagurubandara, E.,N., Ristic, G. and **Podgorski, I.** The Role of Adipocyte-Induced CXCL1 and CXCL2 in Metastatic Prostate Tumor Behavior in Bone. Pharmacology Colloquium, University of Michigan, June 2012 (abstract selected for oral presentation).

J. Herroon, MK, Rajagurubandara, E, Hardaway, AL*, and **Podgorski, I**. Linking obesity with bone marrow inflammation and metastatic prostate cancer: role of adipocyte-derived cathepsin K. 12th International Conference on Cancer- Induced Bone Disease. Lyon, France, November 15-17, 2012.

K. Hardaway, AL*, Herroon, M., K., Rajagurubandara, and **Podgorski, I**. Investigating the Role of Adipocyte-derived CXCL1 and CXCL2 in Prostate Tumor Behavior in Bone Using Long-term Culture System. Tumor Microenvironment Retreat, WSU, December 2012.

L. Hardaway, AL*, Herroon, M., K., Rajagurubandara, and **Podgorski, I.** Bone Marrow Adipocyte-Derived CXCL1 and CXCL2 in Prostate Tumor Progression in Bone. AACR Annual Meeting, Washington DC, April 6-9, 2013.

4. Recent Publications

1. Trivedi, E.R., Harney, A.S, Olive, M.B, **Podgorski, I.,** Moin, K., Sloane, B.F., Barrett, A., Meade, T.J., Hoffman, B.M. Chiral Porphyrazines as Near-Infrared Optical Contrast Agents: Tumor Specific Accumulation In Vivo, *PNAS,* 107(4):1284-8, **2010** PMID: 20080563
2. **Podgorski, I.,** Inhibiting Cysteine Cathepsins in the Bone, New Functions and Off-Target Effects. *Clinical Reviews in Bone and Mineral Metabolism*; 9(2):81-82, **2011** CRBMM
3. Respondek, T., Garner, R., Herroon, M.K., **Podgorski, I,** Turro, C., and Kodanko, J. Light Activation of a Cysteine Protease Inhibitor: Caging of a Peptidomimetic Nitrile by $Ru(bpy)^{2.}$ *Journal of American Chemical Society* 133(43):17164-12167 PMID: 21973207.
4. Herroon, M.K., Rajagurubandara, E., Rudy, D.L., Chalasani, A., Hardaway, A., and **Podgorski, I**. Macrophage Cathepsin K Promotes Prostate Tumor Progression in Bone. Oncogene, 32(12):1580-1593, 2013.
5. Hardaway AL*, and **Podgorski I**. IL-1β, RAGE and FABP4: targeting the dynamic trio in metabolic inflammation and related pathologies. Future Medicinal Chemistry, 5(10),1089-1108, 2013.
6. Herroon, M.K., Rajagurubandara, E., Hardaway, A.L*, Powell, K*, Turchick, A*, and **Podgorski, I.** FA4/IL-1β Axis Links marrow Adiposity with Progression of Metastatic Tumors in Bone (Submitted to Cancer Research on 6/4/13; under review).

3. Other Active grants:

1. "The Influence of Metabolic Syndrome on Prostate Cancer progression and Risk of Recurrence in African American and Caucasian Men." DoD Health Disparity Award; 4/1/09-8/31/12; **Role: Consultant**

2. " Combination of Imaging and Genomic Approaches to Identify Bone and Tumor Responses to RTK-Targeted Therapy for Metastatic Prostate Cancer." NOMIC Award /Karmanos Cancer Insitute; 6/16/11-6/15/14; **Role: PI**

3. Herrick Foundation/KCI – Prostate Cancer Research Initiatives "Racial Disparities, Metabolic Syndrome, Inflammation and Prostate Cancer Outcomes." 10/01/09 – 9/30/14. **Role: Co-PI**

CONCLUSIONS:

The overall goal of this study is to examine the association between metabolic syndrome and related conditions such as inflammation, insulin resistance and oxidative stress, and aggressive prostate cancer (PCa). The study is based on hypothesis that specific biochemical and genetic factors contribute to racial/ethnic disparity in aggressiveness and recurrence of PCa. We are utilizing an invaluable resource of blood samples collected from a large group of African American and European American prostate cancer patients; we are using this resource to explore biochemical and genetic markers of aggressive disease; no study to date has systematically addressed the issue of metabolic syndrome in predicting risk of developing aggressive versus non-aggressive disease and prostate cancer recurrence in any racial group.

We have completed the patient recruitment to this study. Studies of serum markers of newly diagnosed patients are completed and biostatistical analyses are in progress. The tagSNP analyses using DNA samples from all patients recruited to this study have been completed using custom Illumina GoldenGate® panel of 1535 TagSNPs. Biostatistical analyses are in progress. Final results of this study will be included in the Final report which is due to DOD by October 31, 2013.

We expect our studies are likely to identify novel biochemical and genetic markers for detection and prognosis of aggressive prostate cancer, and provide the basis for future validation of these biomarkers as biological targets for improved therapy and/or prevention of aggressive disease.

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